Genomic Investigation of Lupus in the Skin

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Lupus erythematosus is a chronic autoimmune disorder with a protean clinical manifestation affecting virtually every organ including skin, with tremendous variation between patients. This makes it vital to stratify patients on a molecular basis. We used gene microarray technology for large-scale screening combined with bioinformatics to investigate global patterns of gene expression in cutaneous lupus erythematosus to allow further insights into disease heterogeneity. Unbiased clustering exposed a clear separation between cutaneous lupus erythematosus skin and blood samples. Pathway-based analyses of the differentially expressed genes from sample groups within skin and blood showed prominent apoptosis and interferon response signals. Given their well-known role in systemic lupus, the two processes are potentially critical to cutaneous lupus erythematosus as well. We found both coincident and distinct features between systemic lupus and cutaneous lupus erythematosus, as well as several pathways and processes that are specific to the cutaneous disease that offer potential therapeutic choices in the future. Finally, we identified shared cutaneous lupus erythematosus-skin and -blood transcriptional "hot spots" located on the genome that include several differentially expressed genes previously associated with the systemic disease. The differentially expressed genes included in the hot spots with no systemic lupus associations can potentially be targeted in future studies aimed at identifying risk genes related to cutaneous disease.

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INTRODUCTION

Cazenave, who published Biett's work from the Paris School of Dermatology, coined the term "lupus erythematosus" (LE) in 1833 (Mallavarapu and Grimsley, 2007). LE can have an incredibly diverse clinical presentation, with single or multiple organ involvement. The first mention of "discoidal lupus" as a cutaneous form of the disease is credited to Moriz Kaposi in 1872 (Kaposi, 1875). The prevalence of lupus in the population today is estimated at 20–150 per 100,000 persons and is heavily skewed toward reproductive women, as evidenced by the increased prevalence among women in both white (164/100,000) and African American (406/100,000) populations (Lawrence et al., 1998; Pons-Estel et al., 2010). The disease is reportedly higher among African Americans, African Caribbeans, Asians, and Hispanics in the United States, and higher among Asian Indians versus whites in Great Britain, whereas the frequency among blacks is low in Africa (Danchenko et al., 2006). The determinants of variance in terms of organ involvement and across ethnicities and geographies are unknown.

Seventy to 80% of LE patients develop skin lesions at some point in their lives, and in 20% of patient skin lesions are the initial manifestation of disease (Ghosh, 2007; Lee and Sinha, 2006), with 45% of patients with cutaneous LE (CLE) becoming vocationally handicapped (Kuhn et al., 2007). Malar rash, discoid rash, sensitivity to light, and ulcers in the mouth are four diagnostic criteria published by the American College of Rheumatology in 1997 that relate to CLE manifestations (Hochberg, 1997; Tan et al., 1982). Specific lesions in LE have been categorized as subacute cutaneous, acute cutaneous, intermittent cutaneous (LE tumidus), and chronic cutaneous (CCLE) (Gilliam and Sontheimer, 1981; Wenzel et al., 2010). Discoid LE is the most prevalent CCLE (and is designated as CLE throughout this article) (Gronhagen et al., 2011). Symptoms characteristically start as clearly delineated scaly erythematous papules which then evolve into bigger coin-shaped (discoid) plaques accompanied by scarring in the center, shriveling, and hypopigmentation leading to pitted scars around the mouth. Discoid LE has been linked to certain HLA region genes, including HLA A1-B8-Cw7-DR3-DQw1 and the B7-DR2 haplotypes, as well as C2, C5, and tumor necrosis factor on chromosome 6 and IL10 on chromosome 1 (Fowler et al., 1985; Knop et al., 1990; Lee and Sinha, 2006; Lopez-Tello et al., 2007; Millard and McGregor, 2001; Suarez et al., 2005). Despite the large body of literature surrounding lupus susceptibility, the complete complement of risk factors as well as the molecular and genetic basis of disease initiation, progression, and response to treatment in CLE is poorly understood. Additionally, the molecular initiators of disease induction and the exact details of the inflammatory pathways leading to local tissue damage remain unclarified.

The skin may flare independently of the systemic disease and vice versa, although treatments might improve one or the other or both diseases. Many individuals (10–40%) with the cutaneous form of the disease (CLE) transform to the systemic form (SLE), which suggests a common genetic background and disease-related pathways. (Tebbe and Orfanos, 1997). Although dissimilarities between the cutaneous and systemic conditions have been described (Werth, 2007), the degree to which the genetic vulnerabilities to the two conditions are

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Abbreviations: CCLE, chronic cutaneous lupus erythematosus; CLE, cutaneous lupus erythematosus; DEG, differentially expressed genes; LE, lupus erythematosus; SLE, systemic lupus erythematosus

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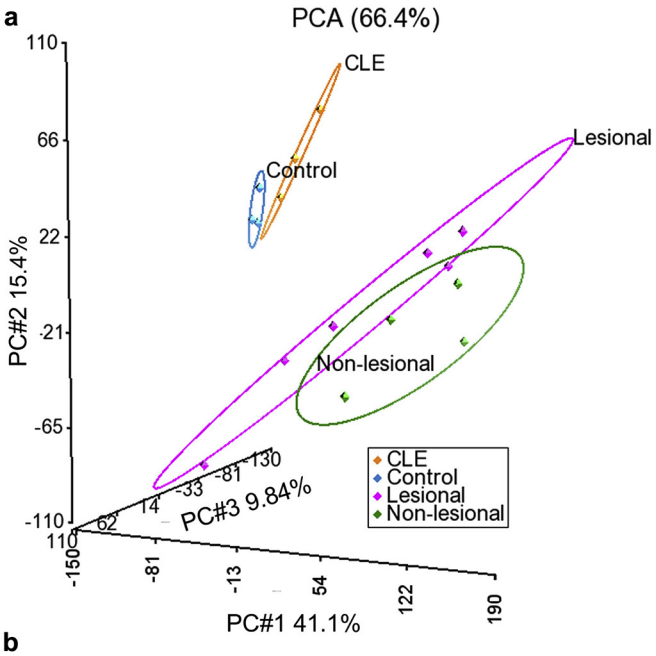

b

Milieu/ Comparison	Total DEGs #	Up regulated	Down-regulated	p-value
Skin Lesional vs. Non-lesional	776	457	319	< 0.05
Blood CLE vs Healthy controls	783	337	446	

Figure 1. **Unsupervised clustering of CLE skin and blood microarray datasets.** (**a**) Principal components analysis (PCA) displays the largest spatial separation based on gene expression variations in the skin and blood sample groups. In the three-dimensional plot, the three principle components, PC#1, #2, and #3 of all probe set IDs and their respective variations are expressed on the x-, y- and z-axes. The total percentage of PCA mapping variability is 66.4%. Each data point represents one sample. The ellipsoids highlight portioning of the different samples. Assignment of samples by color: lesional skin (pink) and nonlesional skin (green) from CCLE patients, blood samples from CLE patients (orange) and healthy control subjects (blue). (**b**) The DEGs in the skin and blood were established according to well-established protocols in the laboratory. Although we noted an 11% overlap in DEGs between the two environments (not shown), most genes in both lists are distinct to the respective environment (skin and blood) from which they are generated. CCLE, chronic cutaneous lupus erythematosus; CLE, cutaneous lupus erythematosus; DEG, differentially expressed gene; ID, identification; PC, principle component; PCA, principle components analysis.

similar or different is unknown. Our genomic investigations comparing genome-wide gene expression in both SLE and CLE is likely to help illuminate the connection between the two conditions. Cutaneous lesions are much easier to procure than lesions from any other internal organs, underscoring the potential of skin to add to the understanding of disease heterogeneity.

Autoimmune diseases are characterized by a complex etiology of interactions between genetic and environment, leading to immune dysregulation. Accordingly, the pathogenesis of LE is considered to be multifactorial and polygenic, with a number of loci likely contributing to variable phenotypic effects. Our studies investigating disease-specific transcriptional patterns aimed to uncover the functional dysregulation associated with genetically determined pathways that underlie the significant heterogeneity of symptoms in cutaneous LE. Identifying such patterns is critical to advancing our understanding of basic mechanisms of disease development and progression and has the potential to define molecular criteria for diagnosis and classification, as well as reveal new targets for therapeutic intervention.

RESULTS AND DISCUSSION

A direct comparison of the skin and blood gene expression data is indicated by the initial unsupervised clustering analysis, where the largest separation is observed between the skin and blood sample groups. The skin samples (lesional and nonlesional) separate out into the "lesional-pathology" signature, whereas the blood samples (CLE and healthy control samples) define the "disease-state" signature (Figure 1). We speculate that variations in gene regulation in the two milieus constitutes the largest contribution to the changes underlying disease pathogenesis. The theme of a prominent apoptosis and type I IFN signature is common to the differentially expressed genes (DEGs) from both skin and blood. All immune-related responses, including natural killer- and dendritic cell-mediated pathways are more pronounced, as well as activated, in lesional skin. This suggests a preferential recruitment and accumulation of these lymphocytes and antigen-presenting cells (innate immune system) at the site of the disease over the systemic milieu. Conversely, breakdown processes such as those related to lysosome/proteasome were more prominent and activated in peripheral blood over the epidermal environment. The similarities and distinctions in gene regulation between the two environments tethers disease etiology and pathogenesis (Figure 2).

Although there were several distinctions in experimental design between our study and previous SLE-microarray studies with regard to tissue type examined (i.e., lesional vs. nonlesional skin biopsy samples of CCLE patients and blood of SLE patients versus healthy control subjects), a total of 152 CLE-skin and -blood DEGs overlapped with previously reported SLE-linked expressed genes or potential risk factors (see Supplementary Table S1 online). Upon mapping the skin and blood CLE DEGs to the canonical Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway hsa05322: SLE, we observed an engagement of several CLE DEGs in all stages, from autoantigen production to the final tissue injury, which in the case of CLE is the skin (Figure 3). Finding common functional annotations between systemic and cutaneous manifestations of LE (e.g., disrupted elements of the complement cascade, heightened immune/inflammatory processes related to type I IFN and chemokines, T-cell and B-cell related pathways, among others) in the skin and blood of CLE patients highlights the mechanistic commonalities underlying the two disease phenotypes. These findings help validate the disease relevance of our data and provide corroboration for suggested disease mechanisms.